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Anti-microbial Studies of Petroleum Ether, Ethyl Acetate and Methanol Extract of Argemone Maxicana Whole Plant, on Human Vaginal Pathogens Causing UTI Infection

FARHEENA SIDDIQUI, PRADEEP SHARMA[1] and MANIK SHARMA[2]

[1]Reader, Chemistry Department,
S.A.T.I. College, Vidisha, INDIA.
[2]Department of Biotechnology,
Principal, Bhoj College, Bhopal, INDIA.

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ABSTRACT

Preliminary phytochemical screening of the leaves extract of *Argemone maxicana* revealed the presence of flavonoids. The ether, methanolic and ethyl acetate extracts of *Argemone maxicana* whole plant has been screened for antimicrobial activities against some human vaginal pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *klebsiella pneumoniae*, *Escherichia coli, B. subtilis, P. vulgaris,Enterobacter, S. choni,* and *Proteus mirabilis* isolated from patient samples. Extracts were found to produce significant inhibition against all the pathogens. Methanolic extract were observed to be more active than ether and ethyl acetate fraction. Extracts are found to be more active against *B. subtilis, Enterobacter, P. aeruginosa,* and *Escherichia coli.*

Keywords: *Argemone maxicana,* Human Vaginal Pathogens, flavonoids.

INTRODUCTION

Today, there is widespread interest in drugs derived from plants. This interest primarily the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects[2,7,14]. *Argemone maxicana* is very common throughout the world with lot of varieties.

Urinary tract infections (UTIs) are a leading cause of morbidity and health care expenditures in persons of all ages. Sexually active young women are disproportionately affected, but several other populations, including elderly persons and those undergoing genitourinary instrumentation or

catheterization, are also at risk. An estimated 40 percent of women report having had a UTI at some point in their lives. Urine located within the urinary tract, excluding the distal region of the urethra is considered sterile in healthy individuals, as indicated by the absence of cultivable bacterial cells. A urinary tract infection (UTIs) describes a condition in which there are micro organisms established and multiplying within the urinary tract. It is most often due to bacteria (95%), but may also include fungal and viral infection[3,11,15].

In the present study petroleum ether, methanolic, and ethyl acetate extracts of *Argemone maxicana* whole plants were screened for potential antibacterial activity toward vaginal pathogens causing urinary tract infections (UTIs).

MATERIAL AND METHODS

Plant materials

The whole plant of *Argemone mexicana* Linn was collected from the local surroundings at Vidisha city of M.P, during the month of November to December 2011. The plant was identified by Dr. S.K. Jain Department of Botany S,S,L, Jain College Vidisha M.P.

Plant were later air-dried, powdered and stored in an air-tight container for further use.

Preparation of extracts

Sample were shattered and screened with 40 mesh. It was soxhlet extracted three times with petroleum ether for 4hr at 60°C. After drying and levigation, the residues were inverse flow extracted at 75 °C with ethyl acetate, then were filtrated and the residue was extracted with methanol at 85 °C for 48hr under reflux condition. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The yield of different extracts was calculated. The powder extracts obtained were then subjected to photochemical analysis to detect the chemical constituents present in each extracts.

Preparation of microorganisms for experiment

All the microorganisms were isolated from in & outpatients samples from Gandhi medical college. For use in experiments, the organisms were sub-cultured in nutrient broth, nutrient agar, Macconky agar and Blood agar media. Muller Hinton agar was used in antibiotic sensitivity testing.

Preparation and application of disks for experiment

(I) Disc diffusion assay

(i) Disc preparation

Sensitivity discs were punched from Whatman No. 1 filter paper, sterilized in Bijou bottles by autoclaving at 121^{o}C for 15mins. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial doubling dilution in Dimethylsulfoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorbed 0.01ml to make the disc potency of 500µg, 1000µg, 2000µg and 4000µg (Akinyemi *et al*., 2005; Vallekobia *et al.*, 2001).

(ii) Inoculum standardization

A loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 – 4mls of sterile physiological saline. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Cheesebrough, 2000).

(iii) Sensitivity testing

Using sterile swab stick, standardized inoculate of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Discs of the extracts and standard antibiotic (Augmentin 30μg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for 30mins for the extract to diffuse into the agar after which the plates were incubated aerobically at 350C for 18 hours. This was followed by measurement of zone of inhibition formed by the test organisms around each of the extract and standard antibiotic discs (NCCLS, 1999).

(II) Agar well diffusion assay

The antibacterial diffusion assay was carried out using Agar well diffusion method as described by Perez *et al*. (1990) and Ahmad *et al*. (1998). One Streptomycin antibiotic drug standard disc (Himedia) of concentration 25 mcg was placed in the centre of each plate as positive control. The assessment of antibacterial activity of the plant alkaloid extract was based on the measurement of diameter of inhibition zone (IZ) in mm formed around the well. Each well was loaded alternately with 100 μl one with 5 mg/ml and the other with 2.5 mg/ml concentration. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings for each concentration and not statistical tools were used to measure the standard deviation.

(III) Micro-broth dilution technique

(i) Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 4000μg/ml, 2000μg/ml and 1000μg/ml. Equal volume (2mls) of extract and Mueller – Hinton broth were mixed. Specifically 0.1ml of standardized inocula (3.3 x 106 CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 350C for 24 hours. Tubes containing broth and leaf extracts without inoculate which served as positive control while tubes containing broth and inoculate served as negative control. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration. That is the lowest concentration that showed no evidence of growth (Akinyemi *et al.*, 2005; Vallekobia *et al*., 2001).

(ii) Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were separately inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35°C for 24 hours and observed. The highest dilution that yielded no bacterial growth was regarded as MBC

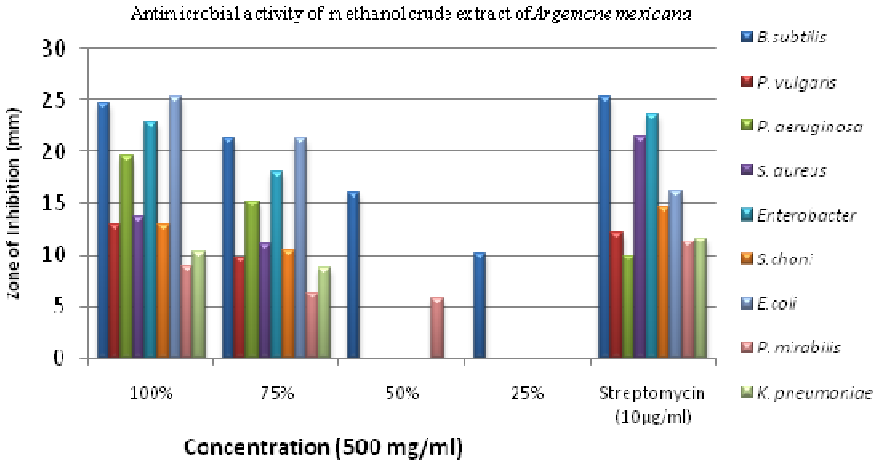
(Akinyemi *et al*., 2005; Vallekobia *et al*., 2001).

OBSERVATION OF RESULTS

Results were recorded as presence or absence of zone of inhibition. The inhibitory zone around test paper disks indicated absence of bacterial growth and it was reported as positive (growth inhibition observed) and absence of zone as negative. The diameters of the inhibition zones were measured in mm (after subtraction the diameter of disc i.e 5mm). The concentration of extract showing inhibition were further diluted and experiment was repeated to identify the minimum inhibitory concentration (MIC). The Percentage of relative inhibition zone diameter (% RIZD) as compare to inhibition obtained from standard drug at same concentration was calculated.

Table 15: Antimicrobial activity of methanol crude extract of *Argimone Mexicana*

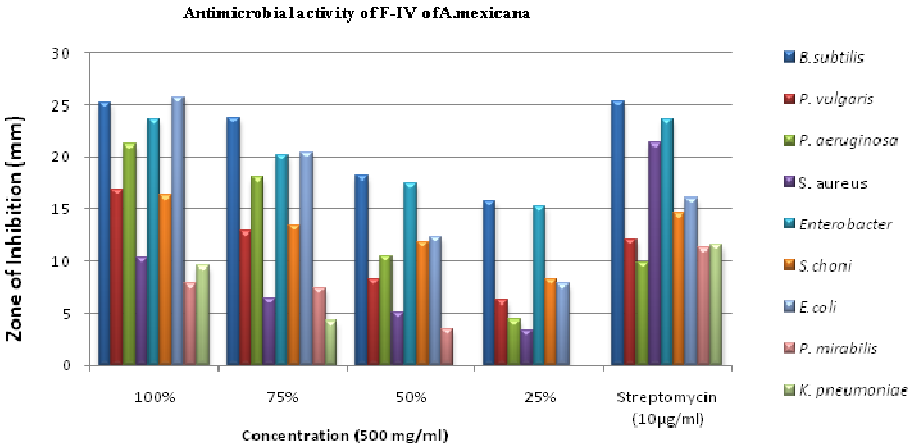
S.No.	Bacteria	Zone of inhibition (mm)				
		Concentration 500mg/ml				Streptomycin
		100%	75%	50%	25%	10µg/ml
1	*B.subtilis*	24.67±0.34	21.29±0.41	16.05±0.17	10.14±0.23	25.32±0.21
2	*P. vulgaris*	12.95±0.12	9.78±0.42	0	0	12.13±0.24
3	*P. aeruginosa*	19.52±0.39	15.23±0.32	0	0	9.9±0.11
4	*S. aureus*	13.72±0.17	11.00±0.23	0	0	21.4±0.14
5	*Enterobacter*	22.91±0.16	17.95±0.25	0	0	23.6±0.22
6	*S.choni*	12.92±0.13	10.5±0.36	0	0	14.6±0.05
7	*E.coli*	25.32±0.12	21.26±0.21	0	0	16.1±0.16
8	*P. mirabilis*	8.96±0.02	6.32±0.24	5.83±0.14	0	11.31±0.12
9	*K. pneumoniae*	10.39±0.01	8.8±0.08	0	0	11.6±0.06



Graph 7: Antimicrobial activity of *Argimone mexicana* on UTI causing bacteria

Table 16: Antimicrobial activity of Column purified fraction IV of *Argimone Mexicana*

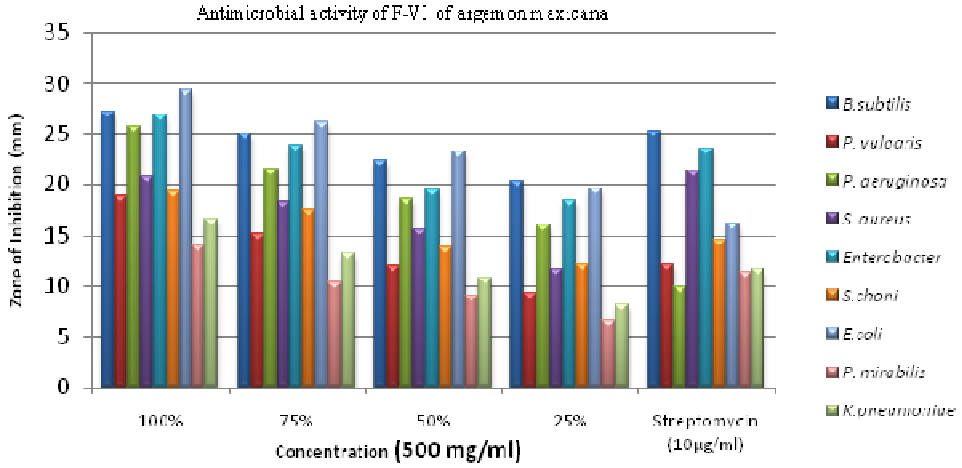
S.No.	Bacteria	Zone of inhibition (mm)				
		Concentration 500mg/ml				Streptomycin
		100%	75%	50%	25%	10µg/ml
1.	*B.subtilis*	25.16±0.11	23.78±0.12	18.24±0.16	15.82±0.14	25.32±0.21
2.	*P. vulgaris*	16.82±0.14	12.84±0.08	8.21±0.06	6.24±0.08	12.13±0.24
3.	*P. aeruginosa*	21.24±0.33	18.08±0.04	10.54±0.04	4.38±0.26	9.9±0.11
4.	*S. aureus*	10.32±0.06	6.44±0.91	5.02±0.01	3.33±0.84	21.4±0.14
5.	*Enterobacter*	23.67±0.12	20.22±0.43	17.47±0.46	15.22±0.08	23.6±0.22
6.	*S.choni*	16.27±0.31	13.41±0.09	11.84±0.04	8.21±0.11	14.6±0.05
7.	*E.coli*	25.7±0.22	20.44±0.08	12.37±0.07	7.81±0.08	16.1±0.16
8.	*P. mirabilis*	7.88±0.24	7.46±0.11	3.54±0.09	0	11.31±0.12
9.	*K. pneumoniae*	9.57±0.33	4.33±0.13	0	0	11.6±0.06



Graph 8: Antimicrobial activity of Column purified fraction IV

Table 17: Antimicrobial activity of Column purified F-VI of *Argimone Mexicana*

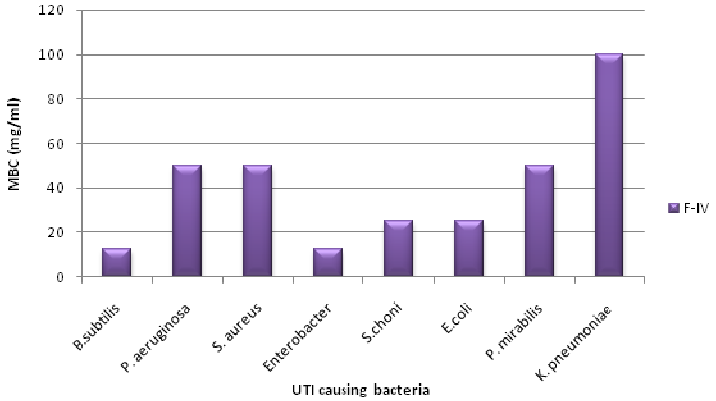
S.No.	Bacteria	Zone of inhibition (mm)				
		Concentration 500mg/ml				Streptomycin
		100%	75%	50%	25%	10µg/ml
1.	*B.subtilis*	27.21±0.02	24.98±0.08	22.43±0.03	20.36±0.16	25.32±0.21
2.	*P. vulgaris*	18.84±0.08	15.12±0.04	11.98±0.23	9.37±0.06	12.13±0.24
3.	*P. aeruginosa*	25.78±0.11	21.51±0.33	18.61±0.52	16.02±0.31	9.9±0.11
4.	*S. aureus*	20.84±0.06	18.33±0.04	15.63±0.24	11.65±0.12	21.4±0.14
5.	*Enterobacter*	26.91±0.13	23.87±0.05	19.54±0.17	18.48±0.08	23.6±0.22
6.	*S.choni*	19.36±0.18	17.52±0.04	13.88±0.06	12.03±0.01	14.6±0.05
7.	*E.coli*	29.32±0.01	26.16±0.22	23.28±0.08	19.65±0.33	16.1±0.16
8.	*P. mirabilis*	13.92±0.41	10.32±0.09	9.01±0.22	6.53±0.06	11.31±0.12
9.	*K.pneumoniae*	16.51±0.07	13.31±0.21	10.81±0.15	8.21±0.31	11.6±0.06



Graph 9: Antimicrobial activity of Column purified fraction VI of *Argimone mexicana*

Table 18: Minimum Bactericidal Concentration (MBC) of fraction IV of *Argimone Mexicana*

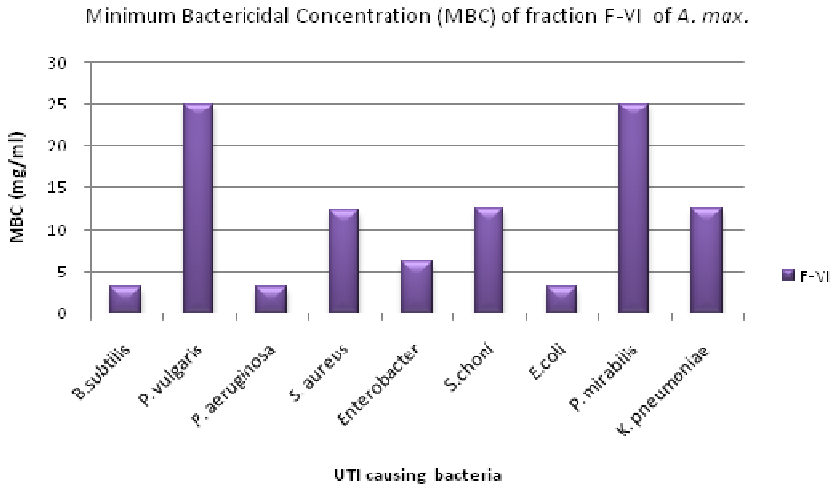
S. No.	Bacteria	Minimum Bactericidal Concentration							MBC (mg/ml
		Concentration (mg/ml)							
		100	50	25	12.5	6.25	3.13	1.56	
1.	*B.subtilis*	-	-	-	-	+	+	+	12.5
2.	*P. vulgaris*	-	-	+	+	+	+	+	50
3.	*P.aeruginosa*	-	-	+	+	+	+	+	50
4.	*S. aureus*	-	-	+	+	+	+	+	50
5.	*Enterobacter*	-	-	-	-	+	+	+	12.5
6.	*S.choni*	-	-	-	+	+	+	+	25
7.	*E.coli*	-	-	-	+	+	+	+	25
8.	*P.mirabilis*	-	-	+	+	+	+	+	50
9.	*K.pneumoniae*	-	+	+	+	+	+	+	100



Graph 10: Minimum Bactericidal Concentration (MBC) of fraction IV of *A.max.*

Table 19: minimum bactericidal concentration (MBC) of fraction VI of A.maxicana.

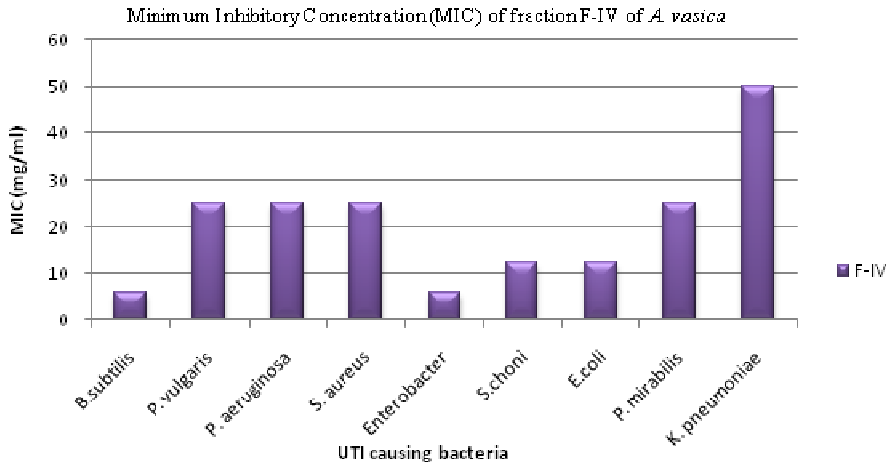
S. No.	Bacteria	Minimum Bactericidal Concentration							MBC (mg/ml
		Concentration (mg/ml)							
		100	50	25	12.5	6.25	3.13	1.56	
1.	*B.subtilis*	-	-	-	-	-	-	+	3.13
2.	*P. vulgaris*	-	-	-	+	+	+	+	25
3.	*P.aeruginosa*	-	-	-	-	-	-	+	3.13
4.	*S. aureus*	-	-	-	-	+	+	+	12.25
5.	*Enterobacter*	-	-	-	-	-	+	+	6.25
6.	*S.choni*	-	-	-	-	+	+	+	12.5
7.	*E.coli*	-	-	-	-	-	-	+	3.13
8.	*P.mirabilis*	-	-	-	+	+	+	+	25
9.	*K.pneumoniae*	-	-	-	-	+	+	+	12.5



Graph 11: Minimum Bactericidal Concentration (MBC) of fraction V of *A. max*

Table 20: Minimum Inhibitory Concentration (MIC) of fraction IV of *A. max*

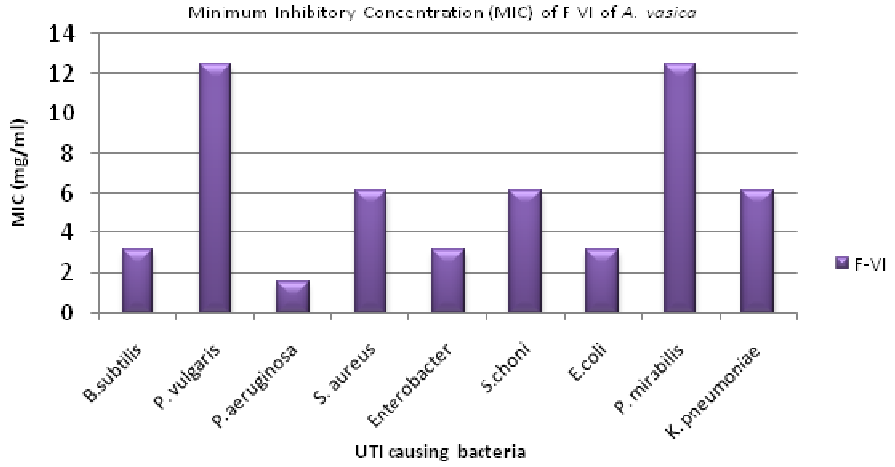
S. No.	Bacteria	MIC (mg/ml)
1.	*B.subtilis*	6.125
2.	*P. vulgaris*	25
3.	*P.aeruginosa*	25
4.	*S. aureus*	25
5.	*Enterobacter*	6.125
6.	*S.choni*	12.5
7.	*E.coli*	12.5
8.	*P.mirabilis*	25
9.	*K.pneumoniae*	50



Graph-12

Table-21: Minimum Inhibitory Concentration (MIC) of fraction VI of *Argimone mexicana*

S. No.	Bacteria	MIC (mg/ml)
1.	*B.subtilis*	3.125
2.	*P. vulgaris*	12.5
3.	*P.aeruginosa*	1.56
4.	*S. aureus*	6.125
5.	*Enterobacter*	3.125
6.	*S.choni*	6.125
7.	*E.coli*	3.125
8.	*P.mirabilis*	12.5
9.	*K.pneumoniae*	6.125



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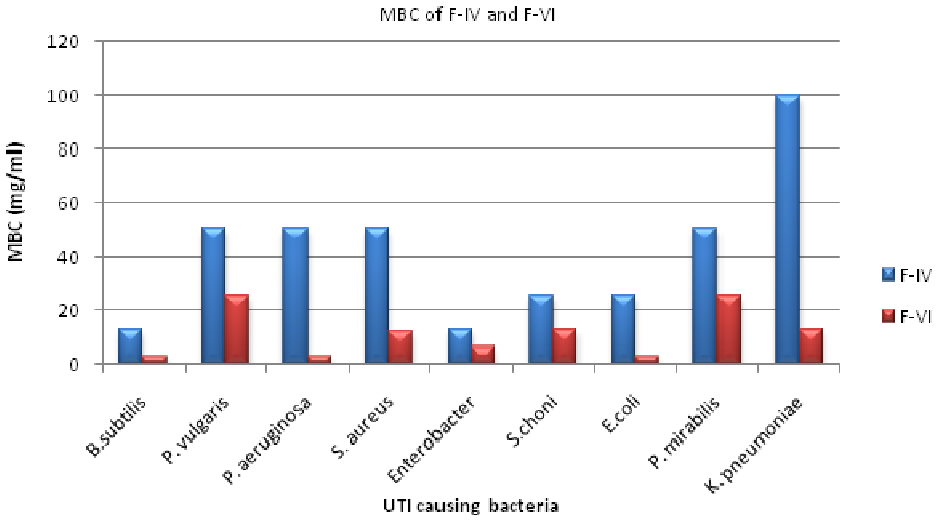
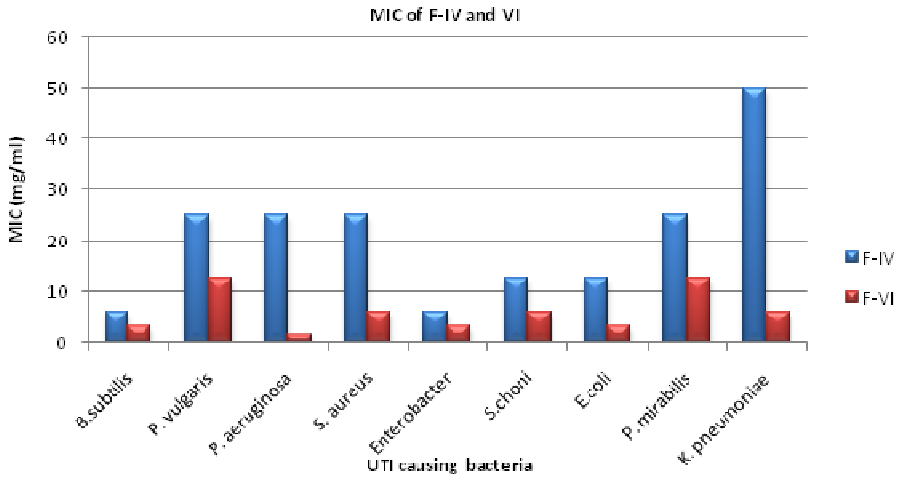


Graph 14: Comparison of MBC of F-IV and F-V fractions of *A.max*



Graph 15: Comparison of MIC of F-IV and F-VI fractions of *A.max*

RESULTS AND DISCUSSION

Argemone maxicana exhibits significant antimicrobial activity against UTI causing microbes. Methanol extract and purified fractions tested against 9 UTI (Urinary Tract Infection) causing bacteria viz. *E. coli, B. subtilis, Proteus vulgaris, P. aeruginosa, S. aureus, Enterobacter, S. choni, K. pneumoneae, P. mirabilis* showed potent antimicrobial activity.

Methanol extract *E. coli* and *B. subtilis* were most sensitive test pathogen to crude extract (500mg/ml) with zone of inhibition 25.32±0.12 and 24.67±0.34 mm respectively **(Table 15, Graph 7).** Column

purified fraction IV and VI were used because of sufficient yield and were found to be more potent than crude extract **(Table 16, 17; Graph 8, 9).** Among the two fractions fraction VI was more effective.

CONCLUSION

From the results of this work, it can be concluded that argemone maxicana has the potential for the production of drug for the treatment of urinary tract infections.

RECOMMENDATIONS

In view of the results obtained in this work, it is recommended that scientists should;

a) Isolate and identify the active compound(s) present in the methanol extract and fractions.
b) Determine the toxicity level of both crude extract and the active compound(s).
c) Screen more plants view of finding alternative treatments to microbial infections

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